

## Opinion

## Leukocyte Adhesion: Reconceptualizing Chemokine Presentation by Glycosaminoglycans

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**Recruitment of immune cells from the vasculature relies on the presentation of glycosaminoglycan-bound chemokines on the luminal side of vascular endothelial cells. However, the current model of chemokine–glycosaminoglycan interactions, and its implications for receptor interactions, remains poorly developed. We propose a refined ‘Chemokine Cloud’ model, arguing that chemokines are not presented to leukocytes bound to glycosaminoglycans, but rather, in solution while sequestered within the hydrated glycocalyx. We posit that glycosaminoglycans provide an immobilized chemokine depot maintaining a ‘cloud’ of ‘solution-phase’ chemokines within the glycocalyx, and that it is this soluble form of any given chemokine that interacts with leukocyte-bound receptors. Our proposition clarifies certain anomalies associated with the current model of chemokine–glycosaminoglycan interactions, with implications for the design of blockers of chemokine function.**

**Chemokine–Receptor Interactions in Transendothelial Cell Migration**

The molecular and cellular events involved in mammalian leukocyte migration can be divided into two major sequential processes: the **leukocyte adhesion cascade** (see [Glossary](#)) followed by the **transendothelial migration** of leukocytes from the vasculature into the surrounding tissue [1] (summarized in [Figure 1](#)). The initial phase of the adhesion cascade relies on interactions between selectins and selectin ligands, which cause leukocytes to roll along the endothelium, despite the rapid blood flow in post-capillary venules. The reduced velocity enables the marginalized leukocytes to sample the endothelial surface for positional cues and to decide whether to re-enter the circulation or commit to transendothelial migration from the vasculature into the surrounding tissue. Crucial to the positional information, which ensures that leukocytes exit the vasculature at the correct point in space and time, are **chemokines**, presented on the luminal side of endothelial surfaces, as well as their cognate **chemokine receptors**, on marginalized leukocytes [2].

Recent mouse-based studies have shown that chemokine receptors represent a key component of the cellular address code that ensures migration of cells to specific destinations under both homeostatic and inflammatory conditions [3]. For example, leukocytes bearing the chemokine receptor CCR10 will recognize its cognate ligands within the vasculature of the skin; leukocytes bearing CCR9 will recognize its ligand in the vasculature of the gut [4]; and CCR7 is an essential marker of cells destined for migration to secondary lymphoid organs, defining it as a component of vertebrate homeostatic leukocyte address codes [5]. Under inflammatory conditions, leukocytes express multiple chemokine receptors and respond to

## Highlights

Recent chemokine receptor structures indicate that oligomerization of chemokines, which is important for chemokine–GAG interactions, is incompatible with receptor binding.

Receptor–ligand structures are incompatible with a model of direct presentation of ligand bound to GAGs.

Recent *in vivo* data suggest that anti-chemokine antibodies targeting chemokines in ‘soluble phase’, rather than attached to GAGs, can be more effective in ameliorating certain inflammatory conditions.

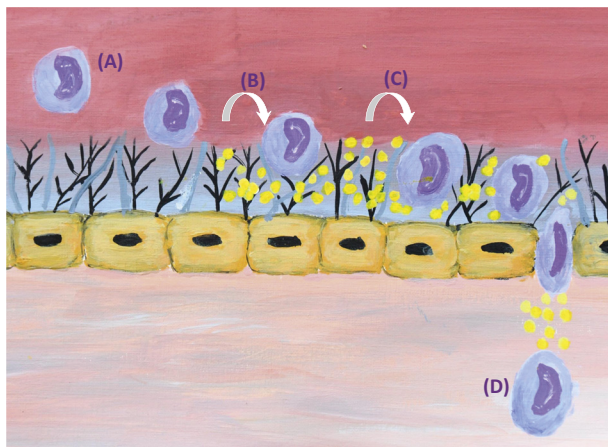
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**Figure 1. The Leukocyte Adhesion Cascade.** This illustration depicts the classic leukocyte adhesion cascade model showing the steps involved in leukocyte recruitment from the vasculature. The steps include: (A) selectin-mediated tethering of leukocytes to the endothelial cell surface; (B) leukocyte rolling along the endothelial cell surface mediated by the transient, and reversible, nature of the selectin-selectin ligand interactions. These interactions enable leukocytes to 'sample' the endothelial surface for cognate chemokine ligands (shown in yellow); (C) leukocyte tight adhesion to the endothelial cell surface mediated by chemokine-dependent inside-out signaling and integrin activation. The activated integrins then bind avidly to their ligands on the endothelial cell surface; and (D) transendothelial migration of leukocytes through the endothelial layer to the subluminal tissue.

locally presented chemokines on the luminal face of endothelial cells in inflamed tissues. In this way, the specificity of cellular homing is achieved.

In both homeostatic and inflammatory contexts, the primary role for lumenally presented chemokines is to mediate '**inside-out signaling**' [1], via their cognate receptors on leukocytes, thereby activating cell surface integrins and facilitating tight binding of the cell to the endothelial surface. This is an essential prerequisite for transendothelial migration, whereby chemokines function specifically to induce high-affinity integrin-dependent binding of leukocytes at the precise point of transendothelial migration.

An essential component of this process is the immobilization of chemokines on endothelial surfaces through interactions with **glycosaminoglycans (GAGs)** [6]. Here, we present a new refinement of the leukocyte adhesion cascade, with a specific focus on chemokine presentation on endothelial cell surfaces. We begin with a description of the ideas that led to the current '**bridge**' model, where chemokine oligomers simultaneously bind to receptors on leukocytes and GAGs on endothelial cells in order to initiate cell adhesion. This is followed by new observations that suggest that the current model is incorrect, and we propose a refined '**cloud**' model. Our new model not only informs our understanding of a crucial aspect of leukocyte homing in health and disease, but also highlights a novel concept that may be central to the development of selective blockers of *in vivo* chemokine activity (including antibodies) for therapeutic purposes.

### Current 'Bridge' Model of Chemokine Presentation in the Leukocyte Adhesion Cascade

An early concern regarding the role of chemokines in the adhesion cascade was how to maintain their local presentation in the presence of powerful **shear flow** in post-capillary

### Glossary

**Axonal pathfinding:** process by which neurons send out axons to interact with the correct targets.

**Beta-sheet fold:** protein secondary structural motif comprising a variable number of connected beta strands between three and ten amino acids in length.

**'Bridge' model:** classic model of direct GAG-bound chemokine presentation to cognate receptors on leukocytes.

**CC chemokine dimers:** CC chemokines form elongated, dimeric structures, with the individual monomers interacting at the N-terminal region.

**Chemokines:** small proteins belonging to an evolutionarily conserved family regulating leukocyte recruitment to, and movement within, resting and inflamed tissues.

**Chemokine oligomerization:** chemokines form electrostatic oligomers, most prominently seen with inflammatory chemokines. These can generate homo- or hetero-oligomers, often giving rise to structures with molecular weights in excess of 1 MDa.

**Chemokine receptors:** members of the G-protein-coupled receptor family that bind chemokines, mediating their biological functions.

**Classical chemokine fold:** chemokine tertiary structure typically incorporates a flexible N terminus, a 3-stranded beta sheet and a C-terminal alpha-helix. The overall tertiary structure is maintained by two disulfide bonds (one in the case of XCL1).

**'Cloud' model:** model proposing that chemokines are not presented directly as GAG-bound entities, but in solution phase at the glycocalyx as a result of dissociation from GAG-maintained depots.

**CXC chemokine dimers:** CXC chemokines form tight, dimeric structures through interactions between the first beta strands of the two monomeric units.

**Evasins:** proteins produced by ticks that bind and neutralize chemokine function. They enable feeding on the host without triggering immune and/or inflammatory responses.

**Filopodia:** finger-like, probing, cellular extensions from cells. In leukocytes, these are involved in

venules, where transendothelial migration takes place. In principle, shear flow precludes chemokines functioning as soluble chemoattractants in plasma, because they would rapidly diffuse away from the source [7]. This concern led to the now widely accepted paradigm that chemokines are anchored to the luminal face of the endothelial layer through high-affinity binding to the GAG chains of cell surface **proteoglycans** [6]. These interactions allow specific chemokines to be presented on endothelial cells at the precise anatomical position where selective leukocyte recruitment is required.

Some years ago, biochemical and *in vitro* analyses of chemokine–GAG interactions provided the first evidence that interleukin (IL)-8 (now referred to as CXCL8) forms a GAG-immobilized **haptotactic signal** on endothelial cell surfaces [8]. Further concrete evidence for this interaction was provided by a study describing the ability of GAG binding-deficient chemokine mutants to recruit cells into the peritoneal cavity of mice [9]. This study showed that these chemokine variants were able to bind and activate their receptors *in vitro*; however, they were unable to elicit cell recruitment *in vivo* [9]. This work, as well as other analyses [10–12], supported the idea that binding to GAGs was a prerequisite for the activity of many chemokines *in vivo*; it also implied that the active form of a chemokine would be that which is bound to GAGs. This concept became an accepted aspect of the leukocyte adhesion cascade model in the field. However, the precise nature of the chemokine–GAG interactions, especially how these facilitate receptor engagement, remained to be defined. Key questions relevant to this mechanism are addressed here.

#### Are GAG-Bound Chemokines Presented to Receptors, or Are Chemokines Donated to Receptors from Their GAG Partners?

Given that chemokines can bind to both receptors and GAGs, it was not clear whether chemokines anchored to GAGs could simultaneously bind receptors (Figure 2) or whether they were released from GAGs and effectively donated to receptors on passing leukocytes. Of relevance to this question are numerous studies demonstrating that, unlike cytokines, such as members of the Fibroblast Growth Factor (FGF) family, which require GAGs for their biological activity [13], chemokines interact with their receptors in the absence of GAGs *in vitro*. Indeed, atomic resolution structures of ternary complexes between FGF, FGF receptor ectodomain, and heparin/GAG exist [14], but whether chemokines also form ternary (GAG–chemokine–receptor) or only binary (GAG–chemokine and receptor–chemokine) complexes is currently not understood. This point is addressed in our refined model, as discussed later.

#### Do GAGs and Receptors Compete for the Same Binding Site on Chemokines?

Many studies have demonstrated significant overlap between the GAG-binding and receptor-binding sites on several chemokines [15–19]. In agreement with such observations, using classical *in vitro* receptor-binding assays, a number of studies have reported the ability of soluble GAGs to compete with receptors for binding of chemokines and, thus, to being capable of neutralizing chemokine function *in vitro* [6]. Structural studies have also shown that the human chemokine XCL1 exists in two distinct structural folds, the **classical chemokine fold**, which is involved in receptor binding, and an entirely different **beta-sheet fold**, which binds to GAGs [20]. Collectively, these studies suggest that chemokine interactions with receptors and GAGs involve the same amino acids on the chemokine surface and, thus, cannot occur simultaneously. Therefore, it seems unlikely that single subunits of chemokines are presented to receptors in the GAG-bound form. The question is, what other models might explain the data?

detecting pathogens, and probably respond to chemokines.

**Glycocalyx:** hydrated and continuous structure on endothelial cell surfaces; rich in proteoglycans, glycoproteins, and glycolipids.

**Glycosaminoglycans (GAGs):**

highly hydrated, branched oligosaccharides; typically extensively sulfated. They form the sugar-rich portion of proteoglycans.

**Haptotactic signal:** chemotactic signal presented in a 2D extracellular matrix or cell surface-bound form.

**Inside-out signaling:** process by which cytoplasmic signaling downstream of ligated receptors (e.g., chemokine receptors) can trigger the conformational activation and clustering of cell surface molecules (e.g., integrins) to mediate high-affinity interactions with their ligands.

**Interstitial:** within the tissue rather than the vasculature.

**Leukocyte adhesion cascade:** model of leukocyte entry from the vasculature into the tissue.

**Proteoglycan:** protein core heavily decorated with GAGs; typically found projecting from cell surfaces; form a key component of the glycocalyx.

**RIP-LCMV mice:** transgenic animals expressing the glycoprotein of the lymphocytic choriomeningitis virus (LCMV) in  $\beta$  cells (islets of Langerhans), driven by the rat insulin promoter (RIP). Infection with LCMV Armstrong results in infiltration of glycoprotein-specific T cells in islets and ensuing autoimmunity against  $\beta$  cells; the symptoms mimic human type 1 diabetes.

**Shear flow:** dynamic flow of fluid within the vasculature, as a consequence of cardiac activity and vessel diameter.

**Transcytosis:** transcellular transport; here, direct movement of chemokines from the subluminal to luminal face of the endothelium surrounding the vasculature.

**Transendothelial migration:** cell movement from the lumen of the vasculature through the endothelial cell layer and into the subluminal tissue.



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**Figure 2. The 'Bridge' Model of Receptor–Chemokine–Glycosaminoglycan (GAG) Interactions.** This illustration depicts the current 'bridge' model in which chemokines (shown in yellow) are presented in a GAG-bound manner to receptors on passing leukocytes. In this model, chemokines bind simultaneously to GAGs on endothelial cell surfaces and receptors on leukocytes. The receptor is depicted as a 7-transmembrane spanning black structure on the leukocyte.

#### Does Chemokine Oligomerization Enable Presentation of Chemokines to Receptors in a GAG-Bound Form?

It has been known for years that most chemokines reversibly oligomerize in solution [21–23]. Chemokine oligomers are also stabilized by their interaction with GAGs, and GAG interactions can promote higher order **chemokine oligomerization** [6,24]. As a highly conserved feature of many chemokines, oligomerization is likely to have some functional significance. That it contributes to the affinity of chemokines for GAGs is now indisputable, as demonstrated by *in vitro* biochemical and biophysical studies [25]. However, given that single chemokine subunits cannot simultaneously bind receptors and GAGs, chemokines have been proposed to oligomerize in order to bind both GAGs on endothelial cells, and receptors on leukocytes, using different subunits of chemokine oligomers [9]. Indeed, this 'bridge' model, whereby chemokine oligomers 'bridge' two cell surfaces, became the paradigm for chemokine presentation in the leukocyte adhesion cascade. Moreover, it has remained in place for some time, despite no concrete evidence of its validity. New observations have now made us reconsider this model.



### New Observations and Considerations Challenging the Current 'Bridge' Model of Chemokine Presentation

A number of observations suggest that the 'bridge' model of chemokine presentation by GAGs to their receptors is not correct.

#### Structures of Receptor–Chemokine Complexes Do Not Support Simultaneous Binding of an Oligomerized Chemokine by a Receptor and a GAG

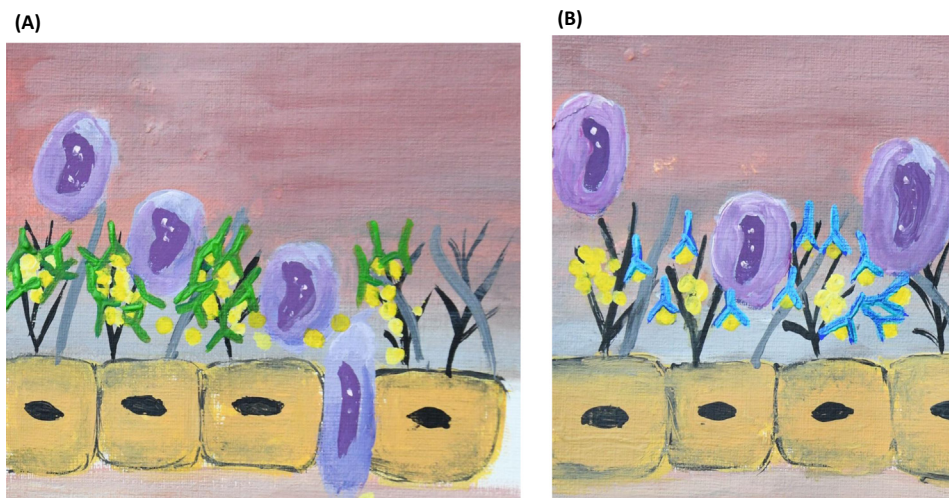
The first clues that oligomerized chemokines could not simultaneously bind receptors and GAGs (the 'bridge' model) were provided by *in vitro* receptor-binding analyses indicating that **CC chemokine dimers** had no measurable affinity for their receptors [26]. When the first structure of a receptor–chemokine complex was solved, the reason became clear: the receptor interacts with chemokine monomers in a manner that mimics the CC chemokine dimer interface and, thus, binding of a CC dimer to its receptor is prevented because of steric incompatibility [27]. Although **CXC chemokine dimers** can bind receptors [28], our recent preliminary results from experimental and modeling studies of CXCR4–CXCL12 suggest that not only does receptor binding to CXCL12 compete with CXCL12 dimer formation, but there also appears to be insufficient binding surface on the chemokine to accommodate both receptor and GAG in a CXCR4–CXCL12 dimer complex. Although these results are preliminary and warrant further validation, the prevailing 'bridge' model does not seem to adequately explain the GAG-based endothelial presentation of chemokines in the vasculature.

#### The *In Vivo* Efficacy of Blocking Antibodies Is Inconsistent with the Concept of GAG-Bound Chemokines Being the 'Active' Chemokine Form

The chemokine CXCL10 is important for T cell recruitment in a range of immune and inflammatory diseases [2] including Behçet's disease [29], type 1 diabetes [30], and certain autoimmune conditions [31]. Thus, it has been identified as an excellent potential therapeutic target in these disease contexts. In a recent study, the ability of two anti-CXCL10 antibodies to ameliorate CXCL10-mediated pathology in the **RIP-LCMV** mouse model of type 1 diabetes was evaluated [32]. While one of the antibodies (clone 1F11) was efficacious in curbing disease symptoms, the second (clone 1B6) was found to be almost ineffective. Further characterization *in vitro* demonstrated that the effective antibody, 1F11, could only recognize free (unbound) chemokine (but not GAG-bound chemokine), while the antibody showing little effect *in vivo* (1B6) recognized both GAG-bound and free chemokines [32]. Of note, the ineffective antibody 1B6 was in fact a more potent inhibitor of CXCL10 in *in vitro* cell migration assays, where all chemokines were in free solution, compared with 1F11. However, since this antibody (1B6) recognized GAG-bound chemokine (the most abundant form), we surmised that most of it would be tethered to the bound chemokine fraction (Figure 3). Furthermore, because the 1F11 antibody that was therapeutically effective in neutralizing the chemokine *in vitro* and *in vivo* had been raised against the free (active) chemokine (and not the GAG-bound form), we concluded that the free form should be targeted for therapeutic efficacy. Nature provides further support for this observation in the guise of inhibitory chemokine-binding proteins produced by hematophagous species, such as ticks. Although requiring full validation, preliminary results suggest that **evasins** from hard ticks [33] are unable to interact with GAG-bound chemokines, yet might be potent inhibitors of chemokine activity.

#### The Glycocalyx Barrier Represents a Conundrum for the 'Bridge' Model

As mentioned earlier, solution-phase chemokine concentrations cannot be maintained in the vasculature due to high shear stress. Moreover, proteoglycans and their GAG chains are not available as free and unrestricted cell surface molecular structures [34,35], a feature that has been essentially ignored in current models and interpretations of chemokine presentation in the



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**Figure 3. Antibodies Targeting Glycosaminoglycan (GAG)-Bound Chemokines Are Ineffective.** (A) Antibodies (shown in green) that recognize GAG-bound chemokines (yellow) are ineffective in preventing recruitment because the GAG-bound form effectively depletes most of the antibodies, leaving little to neutralize the active free form of the chemokine. (B) The binding of antibodies (shown in blue) to solution-phase chemokines in the chemokine ‘cloud’ prevents their interaction with receptors on leukocytes.

leukocyte adhesion cascade. Instead, these highly sulfated and hydrated structures comprise much of the **glycocalyx**, which covers vascular endothelial cell surfaces and shields the vascular walls from direct exposure to blood flow [36]. The hydrated and continuous cell surface glycocalyx layer is an irregularly shaped layer of variable thickness (up to 1.2  $\mu\text{m}$  in microvascular mammalian endothelial cells [37]) that extends into the lumen of blood vessels. Through interactions with the GAG chains of proteoglycans, the glycocalyx sequesters a wide range of enzymes and proteins (including chemokines) that contribute to regulating leukocyte adherence [37].

In the context of GAG–chemokine–receptor interactions, the glycocalyx presents a major conundrum: on passing leukocytes, how do receptors that need to interact with integrin ligands on cell surfaces find their way through this relatively thick glycocalyx layer? Additionally, where on the GAG chains of the proteoglycans within the glycocalyx are chemokines presented? Are they presented at the top or the bottom of this layer, and what implications does their location have for receptor activation and effective integrin–ligand interactions? How is the active ‘free form’ of the chemokine stabilized within the glycocalyx, while still available to receptors on the leukocyte surface, and to neutralizing antibodies?

Careful consideration of the constraints imposed by the glycocalyx along with insights from studies of neutralizing antibodies as well as structural data, led us to propose a refinement of the current ‘bridge’ model of chemokine presentation by GAGs to receptors on leukocytes.

### Chemokine Interactions with GAGs and Receptors: A Refined Model

Our refined model takes into account all of the issues raised earlier and builds on the idea that chemokines, oligomerized or not, are incapable of simultaneously binding to GAGs and receptors. Instead, we propose that chemokines are initially trapped by GAGs in the glycocalyx and then released in soluble form to receptors on leukocytes brought into close proximity by

selectin interactions. The possibility of **interstitial** chemokines being presented to leukocytes as a soluble 'cloud' was initially suggested in an Opinion article by others [38], and we adopt the same terminology to describe our evidence-based refinement of this concept to the vasculature [i.e., the 'Cloud Model' of chemokine presentation (Figure 4, Key Figure)]. The model encompasses concepts described here.

#### Chemokines Form a Soluble 'Cloud' within the Hydrated Glycocalyx Layer

Chemokine localization on GAGs is essential for their maintenance in the hydrated glycocalyx on the luminal face of endothelial cells and in the presence of vascular shear flow. However, for the reasons outlined earlier, GAG-bound chemokines cannot productively interact with their cognate receptors; therefore, freely available, solution-phase, chemokines are the more likely functional units. Why then, is there any requirement of GAGs for presentation of chemokines on endothelial cells? We speculate that the primary role of GAGs is to concentrate chemokines in localized depots near sites of production, particularly under conditions of infection or inflammation. While this is not a new concept, what is new is the idea that these interactions may be dynamic, with chemokines undergoing multiple rounds of binding, disengaging, and rebinding to GAGs to form a 'cloud' of chemokines, effectively partitioned between GAGs and the solution phase within the hydrated glycocalyx. The reversible binding dynamics of the chemokines would enable them to be readily available upon release from GAG chains in order to interact with receptors on passing leukocytes, but multiple cycles of rebinding to GAGs within the glycocalyx would protect them from diffusing away due to shear stress. Direct evidence for this is still required.

### Key Figure

#### The 'Cloud' Model of Receptor–Chemokine–Glycosaminoglycan (GAG) Interactions



**Figure 4.** The model proposes that chemokines within the glycocalyx are present in two forms; one form is bound to GAGs and the other is present in solution phase and comprises the chemokine 'cloud'. This distribution is achieved because chemokines are in equilibrium between the GAG-bound and free form. This equilibrium may facilitate the retention of chemokines in the glycocalyx by transient interactions with GAGs, even in the presence of circulatory flow. It also provides a mechanism for the free chemokine form to bind to receptors on leukocytes.

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### Chemokine Oligomerization May Amplify the Function of the Glycocalyx as a Chemokine 'Sink' and Source of the 'Chemokine Cloud'

GAGs might act as a 'sink', maintaining chemokines in the glycocalyx through electrostatic interactions between the negatively charged GAGs and the basic residues present on all chemokines. Chemokine oligomerization likely plays a significant role in the retention and dynamic release mechanism: multiple binding epitopes on chemokine oligomers and multiple binding sites on GAGs may allow some subunits of the oligomers to disengage GAGs, potentially becoming available to receptors, or to rebind GAGs instead of diffusing away. Similarly, the density of GAG chains and of their chemokine binding epitopes may also contribute to the 'stickiness' of the glycocalyx. In this way, a diffuse chemokine 'cloud' within the hydrated glycocalyx layer could ideally provide a stable source of soluble chemokine, in addition to a localized directional signal for cell migration.

### The Glycocalyx May Support the Formation of Chemokine Microgradients

A microgradient directional signal of chemokines is favored by the production of chemokines from endothelial cells themselves, or from underlying tissues, coupled with the **transcytosis** of the cells to the luminal surface of the endothelium [39,40]. Thus, it is reasonable to speculate that the concentration of chemokines bound to GAGs might be higher adjacent to the cell surface, but lower at the outer reaches of the glycocalyx. While a vertical distribution of chemokines within the glycocalyx has not yet been defined, if this holds true, this arrangement might provide precisely localized microgradients of chemokines between cell surfaces and the outer layers of the glycocalyx. This, in turn, might enable gradient-directed penetration of the glycocalyx by receptor-bearing **filopodia** from leukocyte surfaces, in a manner analogous to **axonal pathfinding** in the developing nervous system. Overall, the relative distribution of chemokines within the glycocalyx might enable chemokine receptors and activated integrin-bearing filopodia to find their way to endothelial cell surfaces, where essential integrin–integrin ligand interactions and subsequent firm adhesion could then take place in the leukocyte adhesion cascade.

### Inflammatory Conditions May Favor Leukocyte Recruitment through Increased Partitioning of Chemokines into the Soluble 'Cloud'

Recent studies have shown that the density of GAG chains affects the affinity of chemokines for GAGs [25,41,42]. Thus, changes in GAG density that alter the composition of the glycocalyx may repartition chemokines between the bound and soluble phase. For example, under certain inflammatory conditions, such as in a proximal microvessel occlusion ischemia rat model [43], GAG chains can be shed from the glycocalyx due to induced expression of proteolytic and GAG-degrading enzymes relative to controls [44]. This, in turn, may facilitate the closer proximity of leukocytes to endothelial cell surfaces compared with steady-state conditions [45,46]. A reduced density of GAG chains as a result of inflammation-mediated shedding might favor the release of more chemokines into the solution-phase 'cloud', subsequently becoming available for activating receptors to initiate the leukocyte adhesion cascade.

### Concluding Remarks

Here, we have presented a reconceptualized hypothetical 'cloud' model of chemokine presentation by GAGs in the leukocyte adhesion cascade. While this model remains speculative, and requires further examination and robust testing, it ties together a large body of data related to chemokine interactions with receptors and GAGs, and the dynamic properties of the glycocalyx. If proven correct, this 'cloud' model could have important implications for the development of therapeutic antichemokine antibodies or other direct antagonists of chemokine

### Outstanding Questions

Are solution-phase chemokines a preferred target for therapeutic antibody development? Antichemokine antibodies have failed in various clinical trials. Would developing antibodies against free, rather than GAG-bound, chemokines be more effective?

How do receptors interpret multiple chemokine cues in the glycocalyx? In inflammation, numerous chemokines are generated. It is not known how these are presented and interpreted by different receptors.

How do membrane-anchored chemokines (e.g., CX3CL1) function within the glycocalyx? Two members of the chemokine family are not secreted but are presented in a membrane-anchored form. It remains unknown how these function within the glycocalyx.

What is the relevance of chemokine oligomerization for function? Oligomerization is characteristic of inflammatory chemokines and enables them to form depots bound to GAGs. Does chemokine oligomerization have other functions?

Integrins activated in response to chemokine binding to receptors have to interact with ligands on the endothelial cell surface. How do receptors bind ligands within the glycocalyx to mediate integrin activation, as well as integrin–integrin ligand interactions on endothelial cells? This process may require breaching the glycocalyx, and may be mediated by filopodia extending from the leukocyte surface.

How do selectins interact with their ligands in the context of the glycocalyx? As part of the leukocyte adhesion cascade, selectins and their ligands also interact on the endothelium. It is unclear how this is achieved within the glycocalyx.

Our model predicts the existence of microgradients in the glycocalyx. Can these be visualized and how do they function?



function. Specifically, our model proposes that such therapeutics should be designed to selectively target solution-phase chemokines (the ‘active’ form of chemokines that bind receptors). Our model also suggests an explanation for the frequent reports of high concentrations of inflammatory chemokines in the circulation of patients with inflammatory diseases, such as psoriasis [47,48] and rheumatoid arthritis [49,50]. We propose that elevated chemokine concentrations might be a consequence of the ‘leaching’ of solution phase chemokines from the glycocalyx cloud into the circulation, which, if correct, could provide a measure of the extent of ongoing inflammation. Our model also provides a putative mechanism by which chemokines, transported to the luminal face of endothelial cells through transcytosis [39], might be presented to passing leukocytes in solution phase within the glycocalyx, and without the requirement for transfer to GAGs. Many questions remain (see Outstanding Questions), but we posit that the proposed ‘cloud’ model merits further investigation to refine our mechanistic understanding of central aspects of tissue-specific leukocyte adhesion and migration in the context of health and disease.

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